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STUDIES ON PNEUMOCOCCI AND STREPTOCOCCI *

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REVIEW OF LITERATURE

The differentiation of the organisms of the pneumococcus-streptococcus group is of importance in the interpretation of blood-culture results, and in the etiologic study of certain infectious diseases, especially endocarditis and rheumatic fever.

The separation of pneumococci from streptococci was first made on a morphologic basis. The tendency of the pneumococcus to grow in pairs of lanceolate cocci surrounded by a well-defined capsule, distinguished it from the streptococcus, which grew in longer or shorter chains of ovoid or flattened cocci without a capsule. Special methods for the detailed study of capsules were devised by Welch, Hiss,[25, 26] Buerger,[8-10, 13, 14] Wadsworth, Rosenow, Medalia, and others. Buerger found that many streptococci encountered in routine work had capsules. He recognized certain types of capsules which he considered diagnostic for pneumococci and Streptococcus mucosus. A comparative study of the various capsular stains by Baehr and Kantor[3] lead to the conclusion that Buerger's method is the most satisfactory and reliable for diagnostic purposes, being succeeded in value by those of Welch and Rosenow.

In 1901, Libman[38] published his observations on the phenomenon of precipitation by streptococci when grown on glucose-serum agar, and the failure of pneumococci to produce this effect. A little later Hiss[25] published his studies on the serum-water medium of Hanna[22] plus inulin, which pneumococci fermented and streptococci did not ferment. The medium of Hanna consisted of 1 part serum to 9 of water, while that of Hiss consists of 1 part serum to 2 of water. Buerger[11] improved this medium, rendering the results more constant and decisive by the addition of 2% Witte's peptone.

Neufeld[50] observed that pneumococci were dissolved in rabbit bile. Levy[37] found that sodium taurocholate dissolves pneumococci and the Streptococcus mucosus grown in broth, but not streptococci. Mandel-

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baum[49] found that ox bile was suitable for the test. Libman and Rosenthal,[42] in 1908, confirmed the differential value of the bile test, and the correctness of the classification of the organisms in use in the laboratory.

Several varieties of streptococci were observed in the many pathologic conditions caused by the chained cocci, and various classifications were introduced. Thus Fehleisen and Rosenbach distinguished the streptococci of erysipelas from those of ordinary infections by certain differences exhibited in the ordinary fluid and solid mediums. Von Lingelsheim distinguished between Streptococcus longus and Streptococcus brevis, depending on the length of the chains, the former supposedly pathogenic, the latter saprophytic. It is now generally conceded that the streptococcus of erysipelas and the streptococcus of ordinary cellulitis are identical, and that the classification based on the length of chains is not at all satisfactory.

In 1903, Schottmüller[65] made an important addition to the cultural methods of differentiation by studying the growth of the organism on plates of human-blood agar. He divided streptococci into 3 groups, thus:

1. Streptococcus longus seu erysipelatos, virulent organisms growing in long chains and producing hemolysis on blood medium.
2. Streptococcus mitior seu viridans, less virulent organisms growing in shorter but often in long chains, and producing green nonhemolyzing colonies in plates of blood agar.
3. Streptococcus mucosus, an organism more closely related to the pneumococcus, producing a transparent mucoid growth with intense green coloration of the blood agar.

Schottmüller found Streptococcus mitior in a variety of conditions, the most important being a group of 7 cases of endocarditis of a chronic infective type which he later designated as "Endocarditis lenta."[66] Similar clinical pictures had been described by Lenhartz,[36] Litten,[44-46] and Harbitz.[23] Lenhartz called the causative agent "Streptococcus parvus"; Litten called it "Streptococcus gracilis." Schottmüller showed that distinctions of size and delicateness of growth were insufficient to characterize an organism of this group, and introduced the blood-plate method of differentiation. In 1909, Rosenow[54] described cases of "chronic septic endocarditis" from the blood of which he obtained organisms he looked on as altered pneumococci. In 1910, Libman and Celler[41] published "The Etiology of Subacute

Infective Endocarditis," in which the cultural characteristics of the organisms causing this clinical entity were fully described and compared with those of pneumococci, hemolytic streptococci, and Streptococcus mucosus, from which they were clearly distinguishable.

The English school, represented by Gordon,[21] and Andrewes and Horder,[2] made extensive studies on the fermentative properties of streptococci from various sources, and classified them according to the source and the carbohydrates they fermented. Thus they described Streptococcus equinus, Streptococcus mitis (in saliva and feces), Streptococcus pyogenes, Streptococcus salivarius, Streptococcus anginosus, Streptococcus fecalis, and pneumococcus. Horder,[32] reported 88 cases of subacute and 18 cases of chronic bacterial endocarditis, in which the causative coccus was classified as Streptococcus salivarius or Streptococcus fecalis. Rarely the influenza bacillus or the pneumococcus was the cause. The pneumococcus cases are doubtful as they had pneumonic complications, and, as Horder said, the infection was most likely a terminal one. Buerger[15] made a critical study of the English biochemical method of classification, using the mediums suggested by Libman as being the optimum for the purpose, namely, fluid mediums containing carbohydrate and unheated serum.* He concluded that "no hard and fast differences in fermentative properties characterize the various groups, the authors preferring to have recourse to the most frequent habitat and to chemical tests. They have failed to use the most favorable medium for growth of the organisms."

An extensive study by Hopkins and Lang,[31] using fermentation methods, yielded an unsatisfactory classification into pathogenic and saprophytic types of streptococci, the latter, however, including organisms from cases of endocarditis. The authors employed mediums without the use of serum and hence their fermentation results are not so reliable as those of Holman.[28-30] The latter, using optimum serum-broth medium for his fermentation tests, and preferring, for valid reasons, qualitative to quantitative tests, classified both hemolytic and anhemolytic streptococci into 8 groups each. The carbohydrates used were lactose, mannite, salicin, and inulin. Studies on capsules and precipitation were, unfortunately, not made. His nomenclature corresponds closely with that of Andrewes and Horder.

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* Studies by Libman, Celler, and Sophian (results presented to the American Association of Pathologists and Bacteriologists in 1913, not yet published) demonstrated conclusively that broths containing carbohydrates and unheated serum constitute the best mediums for the study of acid-production, being far superior to the serum-water mediums suggested by Hanna and Hiss. The serum-water mediums plus peptone give decidedly better results than those without peptone, but are distinctly inferior to the mediums mentioned.

The subject of rheumatic streptococci and of the variation and mutation of organisms will be dealt with later.

Studies in agglutination,* conglutination, precipitins, opsonins, complement fixation, and anaphylactic reactions, while yielding interesting phenomena have not shown sufficiently definite or constant results to be of practical value in the differentiation of the organisms.

METHOD OF STUDY

While most organisms are readily diagnosed by means of a few tests, not infrequently error thus results. Organisms which seem typical when incompletely studied are often found to be atypical when thoroughly observed. Consequently, much of the older literature on this subject is difficult to interpret, for the organisms have not been described in a manner which makes possible a just comparison with modern work. It is of the greatest importance that any organism obtained from the body should be studied thoroughly at first isolation, for there is evidence to show that residence in the blood stream, localization in new parts of the body, that is, in metastatic foci, cultivation on artificial medium, and animal passage may alter the properties of an organism.

1. *Morphology.*—Gram stains are made on spreads of colonies in the blood-culture plates and of the 24-hour growth on 0.5% glucose-serum agar. The relative size, the shape, and the grouping of the organisms are noted.

2. *Capsule.*—The Buerger[8, 9] differential capsular stain is employed. The spread should be made from a fresh culture on a moist slant of 0.5% glucose-serum agar, for this medium is the most favorable for the development and preservation of capsules. We distinguish a streptococcus mucosus type, a pneumococcus type, and atypical forms. Often the capsule is described in detail with special reference to definition, indentation, and staining of the capsular substance.

3. *Glucose Broth 2% (Merck's Peptone).*—The 24-hour growth is observed for turbidity, granular, clumpy, flocculent or mucoid growth, and sediment. Hanging drops are made to determine the length and type of chains formed.

4. *Precipitation Test.*—This consists in inoculating on a slant of 0.5% glucose-serum agar. The ascitic serum for the test should have a specific gravity of not less than 1.013, and should be added to 3 times the volume of 0.5% glucose agar. The slant must be fresh enough to show condensation water at the bottom of the tube. The culture is incubated 24-48 hours. If the organism precipitates, the clear medium becomes hazy, cloudy, or even opaque white in color. Occasionally the whitening is seen only about the condensation water. This effect on the medium is due, according to Libman,[38] to the precipitation of the serum albumin by the acids, chiefly lactic, formed by the action of the organisms on the glucose

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* The classification of pneumococci into 4 strains by means of agglutination phenomena (Dochez) will not be taken up.

in the medium. The test is one of the most reliable and constant for differentiating streptococci, which precipitate, from pneumococci and Streptococcus mucosus, which do not precipitate.

5. *Inulin Fermentation.*—Buerger's[11] modification of the Hiss serum-water 1% inulin medium is used. By the addition of 2% Witte's peptone, more constant and rapid results have been obtained. Tubes are incubated for 4 days, as some organisms ferment the inulin slowly. Some organisms do not ferment the inulin, but cause a decolorization or reduction of the litmus.

6. *Bile Test.*—Two tubes of neutral sugar-free broth containing 1% Witte's peptone are inoculated and incubated until sufficient growth is obtained. To one of these one fifth of the volume of filtered sterile ox bile is added, and the tube is thoroughly shaken. If solution of the growth occurs it usually takes place in a few minutes, but 20 minutes should be allowed, as occasionally a soluble organism may resist the lytic action of the bile for that length of time. The 2nd tube is used for comparison. The objection to this test is the difficulty which is encountered in obtaining sufficient growth of some organisms. The medium used is not a good one for the growth of many of these cocci, and its nutritive properties cannot be enhanced because sugars and serum interfere with the reaction, as shown by Nicolle and Adil Bey. At times it is impossible to perform the test because of insufficient growth. At other times several tubes are inoculated and the sediment from them divided into equal parts for the test.

Libman and Rosenthal[42] found that the lytic action of the bile was not complete, for they obtained growth on blood agar from cultures which had been mixed with bile in 9 pneumococci of 19 tested, and in 3 streptococcus mucosus strains of 12 tested. Of 68 streptococci, none was dissolved, and all gave growth.

7. *Blood-Agar Plates.*—In addition to a study of the appearances of the colonies in the original blood-culture plates, surface inoculations are made with the loop on blood-agar plates with heterologous human blood, and on standard ox-blood plates, prepared according to the method of Bernstein and Epstein.[7] The human blood plates are made in the proportion of 2 c.c. of blood to 15 c.c. of plain agar. Ox-blood plates are made in the proportion of 1 c.c. of defibrinated formalinized ox blood to 10 c.c. of sugar-free agar. A study of the effect of glucose in ox-blood agar plates has shown that the presence of 0.5% and 2% glucose may diminish or entirely inhibit the hemolytic properties of an organism, but may intensify the production of greenish coloration by organisms possessing this property.

The streak cultures on blood plates are observed for 4 days. The manner in which they are examined is of importance, for very different appearances are obtained by direct and by transmitted light. For my observations, the plate was held parallel to and at a distance of about 8 inches from a white background, such as a porcelain slab or a sheet of white paper on the working desk. The eye can look both down on and through the plate in this way. Note is made of the size and shape of colonies, dryness or moisture of growth, color, etc. Some are colorless and transparent, some gray, white, grayish-white, grayish-green, or brownish-green. Changes in the surrounding medium are noted, such as clearing (hemolysis), haziness or precipitation, production of green, or other coloration. Many organisms observed in this way show a linear clearing, which is of no importance. Clearing to be of diagnostic value must be 2-4 mm. in width, and must be produced in 24 hours.

The clearing about a colony was formerly thought to be due to a solution of the hemoglobin of the red corpuscles, and was, therefore, called hemolysis. Similar appearance, however, is seen about foreign particles in blood plates. It is also to be noted that outside the zone of clearing produced by the growth of a colony is a zone of condensation of the blood pigment, and one is led to believe from these 2 observations that the process may be of a physical nature, leading to a driving away of the blood pigment with a heaping up of it outside the clear zone.* Thalhimer notes that intact red blood cells in the medium are essential for clearing, because this phenomenon was not observed in hemoglobin-agar medium.

The cause of green coloration in blood plates was investigated by Ruediger.[63] He observed that some streptococci which clear in plain blood agar grow green in glucose blood agar, and that pneumococci, which grow intensely green in 1% glucose blood agar and moderately green in ordinary blood agar, produce only a faint green in sugar-free blood agar. He concluded that the green coloration produced by bacterial colonies in blood-agar plates is dependent on the production of acid and the action of this acid, probably lactic, on the red corpuscles. He accounted for the varying intensity of green produced by a given organism in different bloods by varying sugar content of the bloods used. This theory cannot be reconciled with the facts obtained from the use of the precipitation test, for pneumococci produce less acid but grow green, while streptococci produce more acid but cause clearing, or may at times grow green. Precipitation, it will be remembered, is due to acid-production, and if blood-plate appearances are due to the same cause, then the results of these 2 tests should parallel one another.

That the presence of glucose has a decided effect on clearing and the production of green coloration is a fact which was observed in these studies. In view of the influence of glucose in the medium it is important in blood-plate studies that the agar should be free of this carbohydrate. The use of agar, which is entirely sugar-free as employed in the ox-blood plates, inhibits the production of green coloration by some streptococci, but does not interfere with the typical growth of pneumococci.

Butterfield and Peabody[17] showed that the pneumococcus produces methemoglobin or hematin by its action on blood. Baerthlein[4] found 3 kinds of changes produced by bacteria in blood mediums: (1) Hemolysis, liberation of blood pigment, stromata of corpuscles left intact.

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* In this connection the phenomenon of concentric clearing is of interest (p. 424).

This occurs in fluid mediums only. (2). 'Hemoglobinopepsie,' liberation and digestion of blood pigment, stromata left intact. This occurs in solid mediums and produces partial clearing with slight greenish discoloration. (3) 'Hemopepsie,' complete digestion of pigment and stromata, the medium being completely cleared. This also occurs in solid mediums.*

Lyall[47] studied the effect of 0.5 c.c. of 18-hour broth cultures incubated for 1 hour with 1 c.c. of 5% washed sheep corpuscles. Those streptococci which caused clearing on blood-agar plates caused complete hemolysis in this test. Those streptococci which produced more or less green coloration on blood plates either caused methemoglobin formation in the cells without solution, or were indifferent in their effect. Krumwiede and Valentine,[35] using this test, did not find a sharp division of anhemolytic streptococci into 2 groups.

Schottmüller[65] in his blood-plate studies, used 2 c.c. of human blood in 5 c.c. of agar, and made both surface and deep inoculations. This is a heavy concentration of blood, and may vitiate some of the results, for I have seen streptococci which caused marked clearing on plates 2: 15, fail to produce any clearing on plates of higher blood concentration. Rosenow adds 0.3-0.5 c.c. of defibrinated human or rabbit blood to 5 c.c. of agar. Holman uses 5 c.c. of defirinated human blood to 100 c.c. of agar.

The method of studying the organisms of the pneumococcus-streptococcus group outlined in this section has been gradually developed at the hospital laboratory under the direction of Dr. Libman, and by means of it there is rarely difficulty in assigning a given organism to its proper class.

CLASSIFICATION OF THE ORGANISMS

While the majority of organisms belonging to the pneumococcus-streptococcus group, as encountered in routine bacteriologic work, adhere to certain well defined types, many observers have met with individual organisms varying from type in greater or lesser degree. Some of these atypical forms partake of the characters of 2 of the type organisms, and may be regarded as transitional forms in the process of evolution.

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* Note the similarity between (2) and (3) of Baerthlein and Types alpha and beta of Smith and Brown.[68]

The organisms of the pneumococcus-streptococcus group are best divided into 4 types, namely:

1. Streptococcus mucosus.
2. Pneumococcus.
3. Streptococcus anhemolyticus.
4. Streptococcus hemolyticus.

Within each group are found variations from type. The 3rd group, in fact, includes such a variety of organisms that a type can hardly be said to exist. This class of cocci has been the cause of much confusion, for it contains bacterial flora found in the mouth, throat, and intestinal tract of man and animals, leading a purely saprophytic existence, and forms culturally indistinguishable from these, but possessing a degrees of pathogenicity for man and animals. While they may be found in localized infections in man, they have seldom been found in the blood stream, except in the disease variously known as subacute and chronic malignant endocarditis, infective or bacterial endocarditis, or endocarditis lenta. The organisms causing this disease have in the course of evolution acquired the property of parasitism. This view was expressed by Andrewes.[1] We may regard such organisms as facultative parasites.

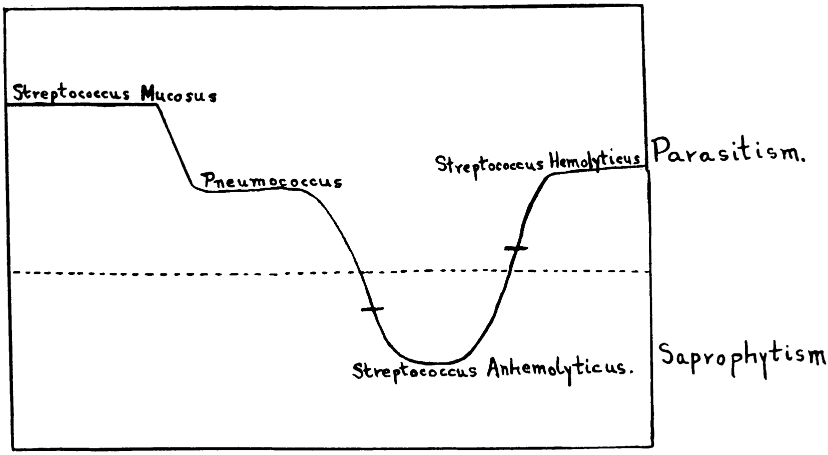

Fig. 1.—Illustrating that there are types and transitions between the types. Note that we have saprophytic pneumococci and parasitic anhemolytic streptococci.

The other 3 groups are far more pathogenic for man and animals. While we meet with them as saprophytes in the mouth, throat, and nasal cavities, we look on them as having great pathogenic potentialities. They are to be considered as essentially parasitic and only facultatively saprophytic. While they have a greater uniformity and fixation of characters than the anhemolytic streptococci, resulting in the production of type organisms, variants may appear under varying environmental circumstances. Moreover, characters acquired as the result of environmental factors may be lost when the particular exciting circumstances cease to exist.

I, therefore, conceive of all these bacteria as forming a continuous line of organisms which may be represented in a graphic manner (Fig. 1). The division into parasitic and saprophytic forms is, of course, only a relative one, for all these organisms can live a saprophytic existence, and all can acquire parasitic properties, but they have a greater adaptation to one or the other mode of life.

TABLE 1

TYPE ORGANISMS

	Gram Stain and Morphology	Buerger Capsule Stain	Glucose Broth	Precipitation	Inulin	Bile Test	Blood-Agar Plates
Streptococcus mucosus	+ Round, ovoid, or biscuit shaped; pairs and short chains	Streptococcus mocusos type	Diffuse turbidity	0	+	Dissolved	Mucoid, green
Pneumococcus	+ Lanceolate diplococci	Pneumococcus type	Diffuse turbidity	0	+	Dissolved	Green
Streptococcus anhemolyticus	+ Round, ovoid, lanceolate, bacillary; pairs, chains, groups	1. Pneumococcus type 2. Atypical 3. None	1. Diffuse turbidity 2. Clumps 3. Granular	+	+ or 0	Not dissolved	Green, gray, white, colorless (slight clearing)
Streptococcus hemolyticus	+ Longer or shorter chains of flattened cocci	1. Atypical 2. None	Granular growth	+	0	Not dissolved	Clearing marked

TYPE ORGANISMS AND VARIATIONS

In Table 1 are described the morphologic and cultural characters of the 4 groups of organisms when typical. As stated, a type does not exist for the anhemolytic streptococci, but I have given in this table the chief variations met with. This group bridges the gap between the pneumococci and the hemolytic streptococci. In Table 2 are noted all the variations met with when these organisms are isolated from the blood, the throat, local foci, etc., and when they have been cultivated on artificial medium, both favorable and unfavorable for the preservation of their type characters.

Streptococcus mucosus has been fully described in the papers of Howard and Perkins,[33] Schottmüller,[65] and Buerger.[13] This organism as indicated in the graphic scheme and in the table of cultural characters seems much more closely related to the pneumococci than to the streptococci. This view is confirmed by the fact that we encounter organisms which grow mucoid and green on blood plates, are dissolved in bile, ferment inulin, and do not precipitate, but show a large typical pneumococcus capsule. Such organisms have been called mucoid pneumococci,* and they occupy a position between the typical Streptococcus mucosus and the typical pneumococcus. I isolated one such organism from the blood of a case of pneumonia with double empyema and purulent pericarditis.

TABLE 2

VARIATIONS FROM TYPE ORGANISMS

	Gram Stain and Morphology	Buerger Capsule Stain*	Glucose Broth	Precipitation	Inulin
Streptococcus mucosus	+ Pairs and chains of twos; round, ovoid, biscuit shaped	**1.** Streptococcus mucosus type 2. Atypical 3. None	(With serum) Diffuse turbidity; mucoid white sediment; fairly long straight chains	0 (rarely positive)	+ (very rarely negative)
Pneumococcus	+ Lanceolate diplococci; short chains; round, ovoid and bacillary forms	**1.** Pneumococcus type 2. Atypical 3. None	Diffuse turbidity; diplococci and short chains; at times long chains	0 (rarely positive)	+ occasionally negative)
Streptococcus anhemolyticus	+ Smaller and larger cocci; single, pairs, chains, irregular groups; round, ovoid, navicular, lanceolate and bacillary forms	**1.** Pneumococcus type 2. Atypical **3.** None	1. Diffuse turbidity 2. Growth of discrete clumps or colonies 3. Granular growth; diplococci, short, long, convoluted and conglomerate chains	+ (rarely negative)	+ or 0
Streptococcus hemolyticus	+ Longer or shorter chains of round, ovoid or flattened cocci	1. Atypical 2. None	Granular growth adherent to side of tube; often a flocculent sediment; short, long, convoluted, and conglomerate chains	+	0 (rarely positive)

* Bold face figures indicate unusual finding.

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* For this reason the name Pneumococcus mucosus, which has at times been used for the Streptococcus mucosus, has caused confusion and had best be abandoned.

I shall now discuss the diagnostic value of each of the tests employed, and note the variations met with.

Morphology.—While one may strongly suspect the identity of an organism freshly isolated from the body when stained by Gram's method, the variations in morphology are so great that no final conclusions can be drawn from observations made in this way. Involution forms are found soon after transplantation, and on prolonged cultivation on artificial medium the whole appearance of an organism in size, shape, and grouping may be altered.

TABLE 2—*Continued*

Variations from Type Organisms

Bile Test	Blood-Culture Plate Plain Blood Agar 2:15	Blood-Culture Plate 2% Glucose Blood Agar 2:15	Surface Inoculations on Standard Plain Ox-Blood Agar Plates 1:10
Dissolved	Surface colonies mucoid, confluent; deepest colonies spread out between medium and plate in a mucoid fashion; intermediate colonies grayish-green; surrounding medium intensely green	Same appearances but production of green pigmentation more intense	Mucoid confluent growth, surrounding zone of intensely green medium, 2-4 mm. wide
Dissolved	Surface colonies ring form; deep colonies grayish-green, surrounding medium moderately green; rarely clearing or concentric zones of clearing	Same appearances, production of green more intense; at times the medium becomes brownish; no clearing	Grayish-green moist growth; zone of gradually deepening green medium, 2-4 mm. wide
Not dissolved	Gray or white colonies, surrounding medium unchanged, slightly greenish or bull's eye appearance; sometimes a zone of clearing, 1-2 mm. wide in 48 or 72 hr.	Greater tendency to produce green pigmentation of medium; bull's eye appearance less marked; no clearing	1. Green 2. Gray 3. White 4. Colorless 5. Linear clearing
Not dissolved	Gray or white colonies surrounded in 24 hr. by clear zone 2-4 mm. wide; rarely concentric zones of clearing	Zone of clearing narrower; clearing may be be altogether absent or delayed	1. White 2. Gray 3. Colorless Wide zone of clearing in 24 hr.

Capsule.—Buerger[11] in his work on pneumococci and allied organisms depended for diagnosis in cases of doubt on the capsule stain, because at that time no organism with a pneumococcus type capsule had been encountered which did not on further study prove to be a pneumococcus or an organism which could be made to revert to a pneumococcus. Since that time, however, anhemolytic streptococci have been isolated from the blood of 2 cases of subacute streptococcus endocarditis (by Drs. Robert Levy and E. P. Bernstein) which possessed typical pneumococcus capsules. I have also found typical pneumococcus capsules on anhemolytic streptococci from other sources, among them strains of 'rheumatic' cocci. Therefore, while in the majority of instances a definite diagnosis of an organism can be made by means of the capsular stain alone, in a small number of instances, notably in the case of intermediate and transitional organisms, it is misleading.

The streptococcus-mucosus type capsule appears as a wide, gelatinous, intensely stained envelope around 2, 4 or more cocci. Its edge is poorly defined and hazy; there is no sharp limiting membrane; there are no indentations between the individual organisms. The pneumococcus type capsule is a fairly wide envelope including diplococci or a short chain. Its edge is sharply defined and deeply stained; there is a distinct limiting membrane. The capsular substance does not stain as intensely as the capsular membrane. The latter follows a course parallel to the outline of the included diplococci, and shows distinct indentations between the individual organisms. From 30-45% of hemolytic streptococci possess capsules of varying appearance. Most often there is a narrow close fitting but well defined membranous envelope which may show no indentations or only slight constrictions between pairs of cocci. The narrow zone of capsular substance stains faintly. Again, the capsule may be ill defined and consist of a fairly well stained envelope without limiting membrane or indentations. When the pneumococcus and Streptococcus mucosus are grown artificially, for some time their capsules may degenerate and become indistinguishable from those seen on many hemolytic streptococci. A full discussion of the types of and variations in capsules of these organisms, with illustrations, may be found in Buerger's articles. Concerning anhemolytic streptococci, I have found that they may have pneumococcus type capsules, atypical capsules, or no capsules.

The 4 strains of Streptococcus mucosus and the 24 strains of pneumococci studied showed type capsules. Of 10 strains of anhemolytic streptococci from the blood of cases of subacute endocarditis, 1 had the pneumococcus type capsule, and 9 were nonencapsulated. Of 12 anhemolytic streptococci from other sources, 2 had the pneumococcus type capsule, 3 had atypical capsules, and 7 had none. Thus, of 22 anhemolytic streptococci, 6, or about 28%, were encapsulated. Of 21 hemolytic streptococci, 7 or about 33%, had atypical capsules. Of 69 streptococci (hemolytic and anhemolytic) studied by Libman and Rosenthal,[42] 32, or about 45%, had capsules. Of 43 streptococci studied by me (21 hemolytic and 22 anhemolytic) only 13, or about 30%, had capsules.

Glucose Broth.—The appearance of the growth in this medium and the type of chain-formation are on the whole of little diagnostic value, for while certain types of organisms behave in a certain way in this medium the variations and exceptions are large in number. Moreover, cultivation on mediums may rapidly abolish the characteristic growth of an organism in fluid medium. Streptococcus mucosus and pneumococci produce as a rule a diffuse clouding of the medium, while streptococci grow in a granular manner. The tendency of the cocci from cases of subacute endocarditis to grow in discrete clumps or colonies in broth is a property which, according to Rosenow, is closely associated with their power to produce embolic endocardial lesions in animals. Of the 10 strains I studied, 7 showed this characteristic to a greater or lesser degree on isolation, while 3 strains produced a diffuse turbidity. Of 12 anhemolytic streptococci from other sources, 9 showed a clumpy or granular growth, while 3 produced diffuse turbidity.

Precipitation.—Of the differential tests, this is one of the most valuable and constant. It separates sharply Streptococcus mucosus and pneumococcus, which produce no whitening on glucose-serum agar, from Streptococcus anhemolyticus and Streptococcus hemolyticus, which produce whitening. Of the 4 strains of Streptococcus mucosus and the 24 strains of pneumococci, none precipitated. Libman and Rosenthal[42] had 2 strains of Streptococcus mucosus which precipitated, one for 4 and the other for 2 generations. Two of their pneumococci, which had been in bile, precipitated for 1 generation. No coccus from a case of subacute endocarditis has been encountered which did not precipitate within 48 hours. Of my 10 strains, all precipitated intensely in 24 hours except Organism B, obtained from Dr. E. P. Bernstein, which possessed a pneumococcus type capsule and whose power of precipitation diminished noticeably after several transplantations. Of the 12 anhemolytic streptococci from other sources, Organism 26496 did not precipitate until the 3rd transplantation and passage through a mouse. All the others of this series precipitated. Of the 21 hemolytic streptococci, all were found to precipitate.

Inulin Fermentation.—Streptococcus mucosus ferments inulin very regularly. Buerger's 16 strains, and the 12 studied by Libman and Rosenthal all fermented inulin. Of the 4 strains I studied, 3 fermented and 1 did not, even after animal passage. The property of fermenting inulin is not constant for pneumococci, as a given organism may ferment in one generation but not in another. On 1st isolation, however, the property is fairly constant. Of 24 pneumococci, 22 fermented strongly and rapidly; 2 fermented slowly and slightly. Libman and Celler[41] state that about one third of the cocci from cases of subacute endocarditis fermented inulin on isolation and retained the property in spite of prolonged artificial cultivation. Of my 10 strains, 7 fermented, 1 reduced, and 2 had no effect. Dr. Celler tested this property in a series of old stock cultures and found that all strains which had previously fermented inulin still did so, and that 2 strains, previously noted as not fermenting, now did ferment. The oldest of these cultures which fermented was about 5 years old. Of 12 anhemolytic streptococci from sources other than endocarditis cases, 5 fermented inulin. Of these 5, 1 lost the property on 4th transplantation, another fermented slightly, and then not at all 2 months after isolation, a 3rd lost the property on 3rd transplantation, but regained and retained it after passage through a mouse. Of the other 7 strains, 1 caused reduction, while 6 had no effect. Of the 21 hemolytic streptococci, none fermented inulin.

The power of the inulin-fermenting anhemolytic streptococci from subacute endocarditis cases to retain the property for years after isolation is very striking. All these observations are based on inulin medium made up with peptone, according to Buerger,[11] as old methods are not reliable.

Bile Test.—When sufficient growth is obtained in neutral broth to perform a fair test of solubility in bile, this is the most reliable means of differentiating Streptococcus mucosus and pneumococcus on the one hand, from anhemolytic and hemolytic streptococci on the other. The 2 former are soluble, the two latter insoluble in bile. But the poor nutritive quality of the medium necessary for the performance of this test and the relative frequenty with which insufficient growth for the test is obtained, makes it unsatisfactory. Whenever a good growth has been obtained the result always followed the rule stated. An organism, 4279, to be discussed in detail later (p. 438), was found on isolation to resemble a pneumococcus except for its insolubility in bile. On cultivation and animal passage it exhibited all the properties of an anhemolytic streptococcus.

Blood Agar Plates.—I have studied the appearances of the organisms in the original plain and glucose blood-agar plates, and when they have been streaked out on the surface of plain ox-blood agar plates, as is seen in Table 2. In addition, I employed surface inoculations on heterologous human blood-agar plates and on 0.5% and 2% glucose ox-blood agar plates. From these studies the following statements may be made:

1. The presence of glucose in the medium diminishes and may entirely inhibit clearing.

2. The presence of glucose in the medium intensifies the production of green coloration. Organisms which do not produce green on sugar-free medium may produce a narrow greenish zone around the growth on glucose blood agar.

3. Clearing to be diagnostic of Streptococcus hemolyticus must appear within 24 hours and should consist of a zone 2-4 mm. wide.

4. Occasionally an organism which causes clearing in homologous blood (that is, medium made up with the blood of the patient from whom the organism is obtained) will not produce clearing on heterologous human blood.

A case of subacute streptococcus endocarditis with embolic gangrene of the leg yielded an organism which cleared in 24 hours in the original blood-culture plates, but which did not clear on heterologous human blood. While this phenomenon had been observed in a case of pneumococcemia and in a case of otitic sinus thrombosis (4319-4334, p. 440), it had never been observed in a case of this kind. These observations emphasize the necessity of employing blood other than that of the patient before making the final diagnosis of an organism. The cells of the patient may be so loaded with the toxic substances produced by the organism, that they break down very readily when additional poisons are formed by the growth in their immediate vicinity, whereas normal cells are not thus handicapped. It would be of interest to grow these organisms on agar made with the blood of another patient suffering from the same disease.

5. Occasionally an organism which clears on human-blood agar will not clear on ox-blood agar.

6. Organisms which produce a green coloration of the medium, when grown in the depth of a plate, frequently do not produce such coloration, when inoculated on the surface.

7. For diagnostic purposes the appearances on plain human-blood agar and sugar-free ox-blood agar are to be relied on. Surface inocu-

lations are more readily interpreted and more decisive than deep inoculations.

Streptococcus mucosus produces a mucoid, gelatinous, confluent, transparent growth, while the medium underlying it and for a considerable area about it becomes intensely green. The pneumococcus produces a moist grayish-green growth surrounded by a zone 2-4 mm. wide, of gradually deepening green medium, outside of which there is frequently a delicate line of clearing on ox-blood plates. Discrete surface colonies may show the ring form described by Buerger.[12] The ring colonies are best obtained by making an emulsion of pneumococci in a small amount of salt solution, and then pouring this over the surface of a glucose serum agar plate. The finding of ring colonies is often of great value in isolating pure cultures. Rarely a pneumococcus is obtained from the blood which clears in the original blood-culture plates, but does not clear in heterologous blood. Libman,[39] in 1905, isolated from the blood of a pneumonia patient a pneumococcus which produced concentric zones of clearing in the original plates. The organisms from the sputum of this patient grew typically green without clearing. Ruediger[62] reported in 1906 that he occasionally met with hemolytic pneumococci. None of the 24 pneumococci which I studied showed any of these variations.

The hemolytic streptococci produce a transparent colorless, a gray, or a white growth, the underlying medium and the surrounding medium for a distance of 2-4 mm. becoming perfectly free of blood pigment in 24 hours. Outside this clear area is a condensation or heaping up of blood pigment. Libman[40] recovered a streptococcus from the blood of a phlebitis patient which produced concentric zones of clearing, outside each of which was seen the condensation of pigment.

The anhemolytic streptococci produce a variety of appearances on blood mediums. Of the 10 streptococci from patients with subacute endocarditis, grown on human-blood agar, 3 produced white colonies, a green zone and linear clearing; 1, pale green growth, a green zone and linear clearing; 3, grayish growth, slightly green zone; 2, grayish white; and 1, gray, with no green pigmentation whatever. Of 9 of these organisms grown on sugar-free ox-blood agar one produced a grayish growth with underlying medium green; 1, transparent growth; 3, gray; 3, grayish white growth; 1, white. Thus 8 produced no green pigment whatever, in contradistinction to the constant production of a well-defined green zone by pneumococci on sugar-free ox-blood agar. Of 4 anhemolytic streptococci from the blood of patients not endocarditic, grown on human-blood agar, 3 produced a dry gray growth, 1 an intensely green growth; while on ox-blood agar 2 produced a gray, and 1 a grayish-green growth. Of 8 anhemolytic streptococci from sources other than the blood stream, grown on human-blood agar, 1 grew gray and transparent, 1 white, 2 dry gray, 2 grayish-white, 1 grayish-white, with green pigmentation of the medium, and 1 intensely green. Of 7 of these grown on ox-blood agar, 2 grew gray and transparent, 1 white, 2 grayish-white, 1 grayish-green. and 1 pale green.

Thus we see that while a number of anhemolytic streptococci produced greenish pigmentation on human-blood agar, and a few on ox-blood agar, all the pneumococci produced a characteristic appearance on ox-blood agar plates, namely a grayish-green growth or deposit of organisms, a zone of gradually deepening green medium 2 to 4 mm. wide, limited by a delicate line of clearing. I wish to call attention to a characteristic appearance in the original plain-agar blood-culture plates of the cocci from patients with subacute endocarditis. There is the nucleus, pin-point to pin-head in size, formed by the white or gray opaque colony, surrounded by a demarcation zone of hazy partially cleared medium, outside of which is an areola of greenish pigmentation, or later on a narrow zone of clearing. The whole resembles the bull's eye of a target in appearance.

Summary.—In general, if an organism does not precipitate, it is either a pneumococcus or Streptococcus mucosus, and the capsule stain and the character of growth on glucose-serum agar and the blood agar determine which of these it is. If an organism precipitates it is almost surely a streptococcus, and the growth on blood agar determines whether it is hemolytic or anhemolytic. But every organism must be studied completely for some irregularity in its characters will reveal the variations taking place, especially among the anhemolytic streptococci.

ANHEMOLYTIC STREPTOCOCCI

Schottmüller,[65] in 1903, distinguished between streptococci which hemolyzed and those which did not hemolyze on blood agar. The latter he called Streptococcus mitior seu viridans. In blood plates, 2 parts of human blood to 5 parts of agar, deep colonies appeared as fine green points hardly pin-head in size. If only a few drops of blood were added to the agar a narrow hemolytic zone appeared. Surface inoculations on blood agar yielded a fine gray or blackish-green deposit. Individual colonies were fine, almost colorless, later gray to greenish-black points. Some strains grew more richly on certain bloods and produced a narrow clear zone in the course of days. Schottmueller obtained such cocci from a great variety of local lesions of the mouth, nose, accessory cavities, and intestinal tract, and from the blood stream in a case of pancreatic necrosis, several cases of pylephlebitis, and 7 cases of chronic streptococcus endocarditis. The endocarditis cases were clinically distinct, and he later called this disease "Endocarditis lenta." Much of his material was obtained post-mortem. His assertion that the organism is specific for endocarditis lenta can not be supported, for Horder[32] and Libman[41] have had cases caused by the influenza bacillus. While his bacteriologic studies lacked satisfactory capsule studies, the inulin, precipitation, and bile tests, he observed that surface cultures on blood agar did not produce as much green as deep colonies, that some strains slowly produced clearing, and that the concentration of blood was a factor.

Rosenow, in 1909 and 1910,[54, 56] described cases of chronic endocarditis caused by organisms which he called pneumococci. They differed from pneumococci of pneumonia cases in producing less green on blood plates, in losing the power to ferment inulin soon after isolation, in growing in clumps in fluid mediums, and in adhering closely to the surface of agar slants. This special character of clumpy and adherent growth was the more marked the more chronic the case, and the later in the course the organism was isolated; and was closely related to the power of producing endocarditis in animals. Artificial cultivation caused these characters to disappear after a variable time, all the strains gradually changing into typical lanceolate diplococci, often encapsulated, growing as typical pneumococci on blood agar and in broth. On animal inoculation the change occurred abruptly. "It has been impossible to so modify strains of Streptococcus viridans which these organisms much resemble." Agglutination studies seemed to show a closer relationship to ordinary pneumococci than to Streptococcus pyogenes and Streptococcus viridans. "The strains should be looked on as belonging to the pneumococcus group, and as having undergone environmental modification, and not to be regarded as strains of Streptococcus viridans." * In 1910, Rosenow[55] stated that

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* Rosenow probably here referred to green-growing anhemolytic streptococci from sources other than endocarditis cases.

not all the strains fermented inulin, and that those which did ferment lost the property entirely after prolonged cultivation on blood agar.* Streptococcus pyogenes, viridans, and salivarius did not ferment. Cocci from animals dying of endocarditis showed the original special characters, while those dying from bacteriemia or pneumonia soon after inoculation showed the characters of typical pneumococci. Reversion to normal type was caused by (1) prolonged cultivation, (2) short residence in normal serum, and (3) animal passage. Ordinary pneumococci grown in endocarditic serum acquired the special characters of endocarditic cocci. Endocarditis serums agglutinated normal pneumococci, but not hemolytic streptococci.

In his 1912 paper on experimental endocarditis, Rosenow[57] stated that while his organisms were identical with Schottmueller's and Horder's, they were to be regarded as pneumococci attenuated and modified by environmental conditions. Recognizing them as different from ordinary pneumococci, he considered them more closely related to typical pneumococci than to typical streptococci.

In 1910, Libman and Celler[41] showed that their cocci from cases of subacute endocarditis could be readily distinguished from hemolytic streptococci and pneumococci, when freshly isolated from the human body. They were small gram-positive, nonencapsulated cocci, all precipitating and resisting the lytic action of bile. One third fermented, two thirds did not ferment inulin.† Those that fermented retained the property over long periods of time. In original blood-culture plates the colonies were white, with or without a green or opaque zone about them. Subsequent cultures might show (1) a production of green pigment, (2) a moist white growth, or (3) a dry, almost colorless growth.

Libman and Celler said:

> At first these organisms, especially those that do not ferment inulin, would appear far removed from pneumococci. But we have seen that pneumococci may lose their capsules, may lose the property of fermenting inulin, and may acquire the property of precipitation. Many of the organisms grow on blood plates like pneumococci. But we have not hitherto met with pneumococci which when isolated from the blood were resistant to the action of bile.‡ If we should meet with such pneumococci, we could more easily suspect that the organisms under discussion are altered pneumococci. The features of the endocarditis cocci have remained constant in subinoculations over a long period of time (in

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* This is contrary to our experience (p. 422).

† Of the 10 strains described in this paper, 7 fermented inulin. This large percentage may be accidental or due to an improved quality of inulin.

‡ Organism 4279 (p. 438) appears to be such.

some instances as long as eighteen months). Every organism that fermented inulin still does so. After repeated animal inoculations (mice, rabbits) no change in the important characteristics have been demonstrated. Capsules have never been developed.

At the International Congress in London, Libman[43] said that he had not been willing to state definitely that the organisms could come from pneumococci, although he believed they might, because he had not seen pneumococci that had become bile insoluble in the body, but having shortly before made such observation (Organism 4279 described in this paper), he believed the organisms in question could come from either pneumococci or streptococci, but that when isolated fresh from the body they were readily distinguished from both.

TABLE 3

STREPTOCOCCUS ANHEMOLYTICUS FROM BLOOD OF CASES OF SUBACUTE ENDOCARDITIS

No.	Gram Stain and Morphology	Capsule	Glucose Broth	Precipitation
B	+ Round, ovoid, bacillary, lanceolate; pairs, short chains, clusters	Pneumococcus type	Flocculent growth; chains 4-8	+ Diminished after 7 transplantations
4110	+ Small, lanceolate and bacillary; short chains, diplococci	0	Discrete colonies; conglomerate convoluted chains	+
4147	+ Small, lanceolate and bacillary diplococci, clusters	0	Diffuse cloud; long convoluted chains	+
4170	+ Small, lanceolate and bacillary diplococci, clusters	0	Discrete colonies; conglomerate masses of chains	+
4206	+ Small, navicular, short chains and clusters	0	Diplococci, short, long and conglomerate chains	+
4275	+ Tiny, in chains and groups	0	Clumps; long convoluted and conglomerate chains	+
4026	+ Medium size diplococci, chains	0	Granular growth, convoluted chains	+
3975	+ Large cocci, diplococci, clusters	0	Diffuse cloud and clumps; diplococci, short chains	+
4342	+ Small, ovoid and lanceolate, chains	0	Diffuse cloud, chains of moderate length	+ 48 hr.
4357	+ Small lanceolate diplococci, short chains	0	Clumps, convoluted chains	+

Eleven of 69 streptococci studied by Libman and Rosenthal[42] were anhemolytic. All were from local lesions. Seven grew white and moist, 3 green, and 1 delicate and dry on blood agar. These organisms, it was stated, would correspond at least in part to Streptococcus mitior seu viridans of Schottmueller. Up to July, 1912, no case of blood stream invasion by anhemolytic streptococci was recorded at the Mount Sinai Hospital, except in cases of subacute endocarditis.

English investigators, especially Andrewes and Horder,[2] found organisms corresponding in many respects to those which other writers had isolated from cases of chronic infective endocarditis in the mouth, throat, and intestinal tract of man. They classified them, employing Gordon's tests, as Streptococcus salivarius, anginosus, and fecalis, but did not assert that the types were definite species. They stated that Schottmueller's Streptococcus mitior corresponded to their Streptococcus salivarius and fecalis. In 24 cases of "malignant endocarditis" they isolated from the blood 2 Streptococcus pyogenes, 1 pneumococ-

TABLE 3—*Continued*

STREPTOCOCCUS ANHEMOLYTICUS FROM BLOOD OF CASES OF SUBACUTE ENDOCARDITIS

Inulin	Bile	Human-Blood Agar Plates	Ox-Blood Agar Plates
+	Not dissolved	Delicate dry growth; medium slight green tinge	
+	Not dissolved	Delicate grayish growth; narrow greenish zone	Grayish-white, linear clearing
+	Not dissolved	White colonies, green zone, linear clearing	Dry gray, linear clearing
+	Not dissolved	White colonies, green zone, linear clearing	Gray, linear clearing
+	Not dissolved	Gray growth	Transparent growth, linear clearing
+	Not dissolved	Grayish-white growth	Grayish growth, linear clearing
Slight fermentation in 48 hr.	Not dissolved	White colonies, green zone, linear clearing	Grayish-white, linear clearing
Reduction	Not dissolved	Pale green growth, green zone, linear clearing	Dry white growth, linear clearing
0	Not dissolved	Dry Grayish-white growth	Dry grayish-white growth
0	Insufficient growth	Grayish growth, underlying medium green	Grayish growth, underlying medium green

cus, 6 Streptococcus anginosus, 4 Streptococcus fecalis, and 11 Streptococcus salivarius. "The first two organisms cause a rapidly fatal type, the others a chronic type which has nothing to do with rheumatism, except that this disease predisposes to infection of the valves by these low grade organisms." In a later paper, Horder[32] reported 88 cases of subacute and chronic bacterial endocarditis caused by Streptococcus salivarius and fecalis. Rarely B. influenzae or the pneumococcus was the cause. The pneumococcus cases may have been secondary terminal infections superimposed on and overwhelming the old infection.

TABLE 4

STREPTOCOCCUS ANHEMOLYTICUS FROM BLOOD OF CASES OTHER THAN ENDOCARDITIS

Number	Disease Associated Organisms	Gram Stain and Morphology	Capsule	Glucose Broth
4049	Aseptic polynuclear plural effusion; 3 colonies, 72 hr.	+ Lanceolate, diplococci, short chains, clusters	Pneumococcus type	Diffuse cloud and flocculi; chains of 4-10
4343	Osteomyelitis of mandible............	+ Small ovoid and lanceolate, clusters of diplococci	Narrow but indented (atypical)	Discrete colonies; chains of moderate length
4316	Postabortive infection with B. coli in blood and uterus	+ Small, round ovoid; diplococci, chains, clusters	0	Diffuse cloud short chains and small clumps
4348 B	Streptococcus mucosus meningitis; blood culture showed 1 colony Streptococcus mucosus, 1 colony of this organism	+ Small chains of diplococci	0	Granular growth; long, convoluted chains

Major,[48] in 1912, reported studies on 6 cases of endocarditis lenta, employing methods similar to ours. The organisms corresponded accurately with those studied by us, although none fermented inulin. He observed that surface inoculations on blood agar produced whitish colonies without green pigmentation, and that deep colonies produced a slight zone of hemolysis outside the green pigmented area. "We have never had sufficient evidence to consider these organisms as altered pneumococci." Tested according to the method of Andrews and Horder, they corresponded in part with Streptococcus fecalis and Streptococcus salivarius.

Recently Kinsella,[34] using the same methods, reported the same results in 12 cases. As 4 of the organisms grew indifferently on blood agar, he preferred the name Streptococcus mitis to that of Streptococcus viridans. Only 2 strains fermented inulin, but the optimum medium for this test was not employed.

The classification based on fermentation reactions, of which Holman's is the most accurate, would seem to be more cumbersome than practical purposes warrant. Holman's studies show no relationship between the various types of anhemolytic streptococci and the pathologic conditions produced; for example, 4 types caused subacute endocarditis. Krumwiede and Valentine[35] concluded from agglutination studies that these streptococci constitute a heterogeneous group, and there is no relationship between interagglutination and fermentation reactions. Henrici[24] could not corroborate the claims for specific organ affinity, nor for definite relationship between virulence and fermentation reactions.

TABLE 4—*Continued*

STREPTOCOCCUS ANHEMOLYTICUS FROM BLOOD OF CASES OTHER THAN ENDOCARDITIS

Precipitation	Inulin	Bile	Human-Blood Agar Plates	Ox-Blood Agar Plates
+	+ Property lost on 4th transplant	Not dissolved	Dry grayish; medium unchanged	
+	0	Not dissolved	Dry gray	Dry gray
+	0	Not dissolved	Intense green	Grayish-green growth, linear clearing
+	0	Insufficient growth	Dry gray growth	Gray growth

In the course of one year's routine blood-culture examinations made during the course of a year, I isolated anhemolytic streptococci in 4 cases which presented no evidence of infective endocarditis. One case, 4343, was an osteomyelitis of the mandible, the infecting organisms coming no doubt from the mouth, where Horder finds Streptococcus salivarius. Another was a case, 4316, of postabortion infection, in which I obtained both from the uterus and the blood B. coli and Streptococcus anhemolyticus. Here the infecting organisms came undoubtedly from the intestinal tract, where Horder finds Streptococcus fecalis. In a 3rd case, 4049, an abacterial polynuclear-celled pleural effusion, 3 colonies of anhemolytic encapsulated streptococci were obtained from the blood. The 4th case, 4348 B, a meningitis due to Streptococcus mucosus, yielded 2 colonies in the blood-culture plates. One colony was a typical Streptococcus mucosus, the other was an anhemolytic streptococcus. I cannot say definitely whether this was a case of mixed infection (the meningeal infection was pure), or a case of mutation of the organism in the blood stream, but I am inclined to favor the latter view.

The finding of such organisms in the blood of patients not suffering from subacute endocarditis was a new experience at the hospital laboratory. It might be feared that this would lessen the diagnostic value of the blood culture in subacute infective endocarditis, but the function of the laboratory is to confirm a diagnosis suspected from the clinical symptoms. Where other sources of bacteriemia can be excluded and where sufficient clinical phenomena are present, the finding of anhemolytic streptococci in the blood still indicates the existence of an infection of the endocardium.

The cultural characters of the 22 anhemolytic streptococci from local lesions, from the blood in cases not endocarditic, and from the blood in cases of subacute endocarditis are detailed in Tables 3, 4, and 5. The following conclusions may be drawn from these data:

1. "Streptococcus viridans" is a confusing name for the organisms causing subacute endocarditis, and should be abandoned. A considerable number of the strains produce no green whatever on blood-agar plates. The organism is not specific for the disease; it may cause other lesions, and other organisms, notably the influenza bacillus, may cause the lesions of the disease.

2. These organisms occur as saprophytes in the mouth, throat, and intestine of man. They may acquire parasitic properties, produce local lesions, or invade the blood with or without infecting the endocardium. They are seldom found in the blood stream, however, except in the cases of subacute streptococcus endocarditis.

3. While the anhemolytic streptococci from the cases of endocarditis exhibit much more uniformity and fixation of cultural characters than those from other sources the limits of variation are so wide that accurate cultural differentiation is impossible. The organisms from endocarditis patients are very rarely encapsulated. A considerable

number ferment inulin rapidly and intensely, and retain that power over long periods of time (surely for 5 years) when artificially cultivated.* The anhemolytic streptococci which ferment inulin but come from other sources do not retain the property so tenaciously.

Complement-fixation studies by Dr. Olitsky with the serums of patients suffering from subacute streptococcus endocarditis have given remarkable results. With antigen prepared from the homologous organism, there is a marked fixation; with the mixed antigens made from a number of organisms isolated from the blood of other endocarditis patients, there is a positive but less marked fixation. There is no fixation with a mixed antigen made from strains of similar anhemolytic streptococci isolated from the throat and local lesion. These facts indicate that anhemolytic streptococci from cases of endocarditis assume certain biologic properties after they have invaded the blood stream and infected the endocardium. This corresponds with the greater uniformity of cultural characteristics which we found among them than among strains of anhemolytic streptococci from local lesions. The relatively long residence of the cocci in the body in cases of endocarditis undoubtedly is an important factor in the development of its more uniform cultural and biologic characteristics.

4. There is no correlation between the type of growth in blood medium, the production of green, etc., and the pathologic condition produced. Some from endocarditis patients are gray, white, or colorless, while some from local lesions produce intense green pigmentation.

5. The organisms may be roughly divided into 4 groups, thus:

(a) Capsules present: Inulin +
(b) Capsules present: Inulin O
(c) Capsules absent: Inulin +
(d) Capsules absent: Inulin O

But here again no constant correlation with the disease produced can be discerned. The cocci from subacute endocarditis belong, with a few exceptions, to Groups c and d.

6. The common properties of anhemolytic streptococci are these: They are chain-growing cocci, gram-positive, insoluble in bile, precipitate, and do not cause clearing in 24 hours when streaked on plain blood-agar plates.

In view of these facts I believe that the various names given to these organisms by authors should be discarded for the present, and that we should speak of them simply as Streptococcus anhemolyticus. In comparative studies of Streptococcus anhemolyticus isolated from cases of subacute endocarditis and of Streptococcus anhemolyticus found in connection with some cases of polyarthritis, we may speak of the former as endocarditis streptococci (anhemolytic), and of the latter as arthritis streptococci (anhemolytic).

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* This is in striking contrast to the observations of Rosenow (p. 426).

VARIATION AND MUTATION

Ruediger,[64] in 1906, reported that 2 hemolytic streptococci grown in glucose broth for 2 years had lost the property of clearing blood agar. Buerger and Ryttenberg,[16] in 1907, observed changes occurring in pneumococci in the human body. From the blood of a patient with puerperal infection, they obtained an encapsulated hemolytic organism which 'reverted' to a pneumococcus when passed through white mice. An abscess of the eye developing in this case yielded an organism with pneumococcus capsule and streptococcus cultural characters which could not be reverted. An atypical organism was isolated from an acute osteomyelitis; a 2nd operation some time later yielded typical pneumococci. Here, reversion had taken place in the body. They found that pneumococci may acquire streptococcus properties in the human blood or exudate; that such organisms may revert in the body or in animal passage; that the acquired properties may become fixed by metastasis or by cultivation on mediums; that not all atypical strains can be reverted, and that not all the organisms from 1 case may permit of this change. The cultural studies lacked systematic blood-plate observations, and the bile test was not yet in use. The authors relied on the type capsule as the criterion for differential diagnosis, but said that others might look on their original organisms as atypical streptococci. In the absence of the bile test and accurate blood plate studies, no definite opinion may be formed, but I am inclined to look on 2 of their organisms as hemolytic and anhemolytic streptococci (Case 1 and Case 2) which they succeeded in changing into pneumococci.

Rosenow,[58] in 1912, reported that cultivation on blood agar changed the streptococci of epidemic sore throat into hemolytic streptococci, but that 1 soaking in sterile unheated milk reverted them. A strain grown for 6 months on blood agar reverted to original type on passage through 2 guinea-pigs. A strain grown in milk and passed through a guinea-pig resulted in growth resembling S. mucosus. Hemolytic streptococci from scarlet fever when grown in unheated sterile milk could be made to acquire capsules and to lose much of their hemolytic property. He also noted at this time that drying of the medium with salt concentration made the epidemic organisms hemolytic.

Davis,[20] in 1913, converted a hemolytic streptococcus into a mucosus by passage through a guinea-pig. Intravenous injection of a rabbit with St. mucosus produced suppurative arthritis, from which a hemolytic streptococcus was obtained.

Rosenow[60] in his paper on "Transmutations within the Streptococcus-Pneumococcus Group" reviewed the literature on this phase of the subject, and presented his own experiments. He asserted that Streptococcus hemolyticus, viridans, rheumaticus, the pneumococcus, and Streptococcus mucosus are so closely related that one may change into the other under proper conditions. His methods consisted of prolonged cultivation on blood agar, drying of the medium, varying the salt concentration of the medium, use of oxygen pressure, symbiosis with B. subtilis, and animal passage. Not only did he find that the organisms change culturally, but also that their characteristic serologic reactions and their characteristic "elective affinity" for definite structures in experimental animals are exhibited in their new guise. The organisms are changed not only morphologically and culturally, but biologically as well. These studies have apparently demonstrated relationships which various authors have suspected from isolated observations .

I had the opportunity to study with Dr. Libman a number of organisms submitted by Dr. Rosenow. Some of these represented strains before and after mutation had been effected. Our cultural studies led us to conclude that Dr. Rosenow's criteria for diagnosis were the same as our own.

Two criticisms of the mutation studies have been made. The 1st has been made by upholders of the "Pure Line Concept." Cole and Wright[18] point out that since bacteria are asexual and do not propagate by amphimixis, a pure line consists of the descendants of any single cell, constituting a clone. Within species are cultural races or varieties, each with its own characteristics and range of variability, which exist side by side independent of environment. Each strain transmits the impress of its recent history.*

According to the exponents of the theory mutations previously reported can be accounted for by the presence of 2 or more strains in the original culture. Under varying conditions, one or the other strain predominates in subcultures. If the conditions are maintained for a short time only, 1 strain is suppressed and when original conditions are restored, this strain reappears, gains predominance, and produces an apparent reversion. If, however, the adverse environment persists for a longer time, the less favored strain dies off and cannot reappear when original conditions are restored, thus producing an apparent permanent mutation. This contention casts doubt on our current bacteriologic methods of obtaining pure cultures, and would seem to necessitate the use of Barber's method for the cultivation of single organisms.

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*Observed differences due to slight variation of medium are not true variations. True protoplasmic variations under identical conditions are of 2 kinds: (a) due to causes operating within the cell; for example, unequal division of nuclear material. These are usually minute "fluctuating variations," rarely "mutations or sports;" (b) impressed variations (remembering the tremendous number of generations produced by bacteria in a short time).

TABLE 5

Streptococcus Anhemolyticus from Sources Other than the Blood Stream

Number	Source	Gram Stain and Morphology	Capsule	Glucose Broth
24862	Sputum; lung abscess	+ Small short chains	Close fitting atypical	Flocculent growth; chains of 4-12
S 41	Excised tonsil	+ Small lanceolate diplococci, short chains, groups	Pneumococcus type; lost on transplantation	Granular growth; long convoluted conglomerate chains
26911	Pelvic abscess	+ Moderate size lanceolate and navicular, groups	Narrow, indented, atypical	Diffuse cloud; diplococci; chains of 8-12
C	Sore throat	+ Small navicular, diplococci, clusters	0	Granular growth; long convoluted chains
26941	Bile, acute cholecystitis	+ Large, round, irregular, grouped	0	Granular growth; long chains
26496	Sputum	+ Small diplococci in clusters	0	Granular growth; long and short chains, clumps
26891	Bile; carcinoma of liver and pancreas	+ Moderate size lanceolate and navicular; irregularly grouped	0	Granular growth; short chains and clumps
26942	Urine	+ Lanceolate	0	Clumpy growth; chains of moderate length

Holman's experiments[28] on symbiosis of streptococci with other varieties, pneumococci, and other bacteria lend support to these contentions. He points out that plating from blood agar to blood agar without preliminary growth in serum broth is very apt to give impure colonies.

The 2nd objection has been raised by Holman, who has demonstrated that animal passage as a means of producing mutations is a very fallible method. Spontaneous infection with various types of streptococci and with pneumococci occurred in guinea-pigs untreated or injected with other bacteria. He believes that many of Rosenow's transmutations in animals were secondary invasions from the animals themselves.

TABLE 5—*Continued*

STREPTOCOCCUS ANHEMOLYTICUS FROM SOURCES OTHER THAN THE BLOOD STREAM

Precipitation	Inulin	Bile	Human-Blood Agar Plates	Ox-Blood Agar Plates
+	+	Not dissolved	Dry gray	
+	Slight, 0 2 months later	Insufficient growth	Delicate, gray transparent growth	Delicate, gray transparent growth
+	0	Not dissolved	Dry gray	Grayish-white
+ 48 hr.	+	Not dissolved	Intense green	Pale green growth, green zone, linear clearing
+	+ 48 hr.; lost on 3rd transplant; property recovered by passing through mouse	Insufficient growth	Grayish-white	Grayish-white
0 at first + on 3rd transplant and mouse injection	0	Not dissolved	Grayish-white growth, medium green	Grayish-green growth
+	Reduced	Not dissolved	White	White
+	0	Insufficient growth	Grayish-white	Grayish, transparent

Among the 71 organisms studied by me, many minute variations due to changes in the medium were observed, but they were temporary in nature. Some permanent variations occurred, such as loss of capsule, loss of power to ferment inulin, loss or acquisition of precipitation, etc., but these changes were not sufficient to change the classification of the organisms according to my standards. Two definite mutations were encountered and the detailed observations follow:

Organism 4279 was isolated from the blood* in a case of brain abscess and sinus thrombosis following otitis media, on March 30, 1913. Its characters were as follows:

A gram-positive coccus was seen in pairs and chains; lanceolate, navicular, and bacillary forms.

The colony from original blood-culture plate showed only suggestion of a capsule, but the transplant on fresh 2% glucose-serum agar showed a well defined, indented capsule, approaching the pneumococcus type.

Glucose broth gave a clumpy growth; conglomerate masses of chains.

There was no precipitation.

Inulin fermented in 48 hours.

It was not dissolved in bile.

Human-blood plates gave delicate pin-point grayish-white colonies, with wide green zone and linear clearing. Ox-blood, grayish-green growth, the medium unchanged.

It was difficult to classify this organism for its morphology, capsule, fermentation of inulin, lack of precipitation and green growth spoke for the diagnosis of pneumococcus, while the atypical growth on ox-blood agar, the clumpy growth in glucose broth, and its insolubility in bile allied it to the anhemolytic streptococci. If the organism had been soluble in bile, I should have considered it a slightly atypical pneumococcus. Was I perchance dealing with an organism in the very process of changing from a pneumococcus into a streptococcus, the first evidence of which change was its insolubility in bile?

On its 3rd transplantation (at 4- or 5-day intervals), on 2% glucose-serum agar, precipitation of the medium appeared.

April 16, 1913. The growth from 1 slant was injected into the peritoneal cavity of a mouse, and the animal killed 24 hours later. Culture of the slight exudate gave an organism with a wider, more typical pneumococcus capsule, causing precipitation not only of 2% glucose-serum agar, but also about the condensation water of 0.5% glucose-serum agar. Otherwise it remained unchanged.

April 24, 1913. The culture from the 1st mouse was injected into a 2nd mouse. The organism obtained showed a wide typical pneumococcus capsule, precipitated on 0.5% glucose-serum agar, but did not ferment inulin. Otherwise it remained unchanged.

The organism now corresponded in every way to a green-growing anhemolytic streptococcus, possessing a pneumococcus-type capsule, insoluble in bile and not fermenting inulin.

May 3, 1913, the original organism and the 2 strains from the mice were all found not to ferment inulin.

May 18, 1913, the 3 cultures were studied completely and were now found to have no capsule, precipitated, did not ferment inulin, were not soluble in bile, and grew grayish-green on ox-blood agar. They had lost their capsules and their power of fermenting inulin, and, therefore, corresponded with the anhemolytic, nonencapsulated streptococci.

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* It is of importance to note that only 1 colony in 1 blood plate was obtained. This excludes the possibility of 2 strains being present from the outset.

The view might be taken by some that the organism was an anhemolytic streptococcus which had acquired certain pneumococcus characters in the human body, and which on cultivation and animal passage had reverted to its original type. But I regard this organism as a pneumococcus which, when isolated from the body, had already begun to change into a streptococcus, as evidenced by its insolubility in bile. It would seem that this change in reaction to the bile test is the first to occur when mutation takes place and is, therefore, of biologic importance. Then in their turn came the acquirement of the property of precipitation, the loss of power to ferment inulin, and finally the loss of the capsule. The latter was maintained for some time because of animal passage, but soon disappeared on artificial cultivation.

Two points deserve emphasis: (1) I was dealing with a pure line; (2) the final change was the same with the original strain kept on culture mediums as with 2 strains after passage through 2 different mice. These observations answer the 2 objections which have been made against mutation.

Organism 4319 was isolated from the blood in a case of otitic sinus thrombosis, on April 30, 1913. Its characters were as follows:

A very small gram-positive coccus was seen in pairs, short chains and groups like staphylococci. No capsule enveloped the organism.

Glucose broth gave sparse growth. Short chains and large clumps were observed.

Precipitation was present.

Inulin was not fermented.

It was not dissolved in bile.

Original blood-culture plates showed small grayish-white colonies surrounded by a wide zone of clearing.

Streak cultures were intensely green on plain blood agar, made up with another human blood.

There was a grayish-green growth with only linear clearing on ox-blood agar.

This organism was classified as an anhemolytic streptococcus which had acquired the property of hemolyzing on the blood of the host.

On May 13, 1913, a 2nd blood culture, 4334, was taken because, in spite of operation, arthritic symptoms and cutaneous manifestations of acute 'malignant' endocarditis had appeared. The organism now showed the same morphology, no capsule, precipitated, did not ferment inulin, and was insoluble in bile. In glucose broth, it produced a granular growth with long convoluted chains. On blood-agar plates, it not only hemolyzed the host's blood, but heterologous human blood and ox blood as well.

This streptococcus, originally anhemolytic, acquired first the power of hemolyzing the host's blood, and then as it became engrafted on the endocardium, it acquired the property of hemolyzing other blood as well.

Organism 4348 B. A blood culture was made in a case of streptococcus mucosus meningitis. Two colonies were obtained, 1 of which was a typical S. mucosus. The 2nd was a nonencapsulated gray-growing streptococcus (Table 4).

The culture was taken a few days before death, and this organism cannot be regarded as an antemortem invader. I am inclined to regard it as a mutation taking place in the body. Injection of mice with cultures gave no results.

In our experience we have observed, therefore, the following mutations:

1. Encapsulated hemolytic streptococcus converted into a pneumococcus (Buerger and Ryttenberg, Case 1).
2. Encapsulated anhemolytic streptococcus converted into a pneumococcus (Buerger and Ryttenberg, Case 2).
3. Pneumococcus changed into anhemolytic streptococcus (Organism 4279).
4. Anhemolytic streptococcus converted into a hemolytic streptococcus (Organism 4319-4334).
5. Streptococcus mucosus changed into an anhemolytic streptococcus (Organism 4348 B).

We believe that mutations occur in nature, that they occur uncommonly, and that their occurrence is not of sufficient frequency to interfere with current bacteriologic methods, or with the practical application of those methods to clinical medicine.

Smith and Brown[68] say: "Spontaneous changes in cultural characters do not proceed rapidly enough, if they go on at all, to interfere with current bacteriologic methods."

THE RHEUMATIC COCCI

The literature of investigations into the etiology of rheumatism (acute rheumatic fever, acute polyarthritis) may be found in the writings to be mentioned, especially in "Researches on Rheumatism," by Poynton and Paine.[52] I wish merely to present brief descriptions of the better known organisms believed to be the etiologic factors of the disease.

The diplococcus rheumaticus of Poynton and Paine is described by the authors as follows:

> Minute cocci in pairs, averaging 0.5 micron in diameter.
>
> Stains readily with anilin dyes but does not retain Gram's stain with great tenacity. Stains best in tissues with carbol-thionin.
>
> As a rule, it shows no capsule, but in human tissues an appearance of capsulation may be noticed occasionally.
>
> In liquid media they grow in chains of varying length. In solid media they grow in masses that resemble the arrangement of staphylococci.
>
> They grow aerobically but better anaerobically. Best medium consists of milk and broth acidified by lactic acid.
>
> On blood agar minute white colonies appear in 24 hours. These tend to remain discrete and to alter the blood pigment to a rusty brown color.

Walker and Ryffel[76] and Beaton and Walker[5] found that similar organisms which they isolated in cases of rheumatism grew well in alkaline medium, as described by Wassermann, and produced large amounts of acid, chiefly formic. They also first described the rusty brown or chocolate color produced on blood agar, which they attributed to the formation, by reduction, of hemochromogen. Beattie[6] obtained a micrococcus from 2 joints, but reported no studies on their cultural characters.

Shaw,[67] studying cultures from Wassermann, Poynton and Paine, and Walker concluded that the 3 organisms were identical, and the cause of acute rheumatism.

Andrewes and Horder[2] found Poynton and Paine's diplococcus rheumaticus to be a streptococcus salivarius, and Beattie's diplococcus to be a streptococcus fecalis when subjected to Gordon's tests.

Rosenow,[55] in 1910, stated that Micrococcus rheumaticus might or might not ferment inulin, and grew green on blood plates. Major,[48] in 1912, stated that his cultural studies of Poynton and Paine's No. 34, Beattie's 254, and Lintz's 399 showed no essential differences between them and the organism of 'Endocarditis lenta.'

Various views are held as to the relationship of rheumatism to "malignant endocarditis." Thus Poynton and Paine believe that rheumatism may cause both simple and malignant endocarditis. In simple rheumatism the blood rarely yields the diplococcus because the organisms are localized in closed cavities, such as synovial, pericardial, etc., and not being on the surface of the heart valves are destroyed by phagocytosis and sclerosis. In the necrotic material constituting the vegetations of simple rheumatic endocarditis, the infection lurks quiescent, and is as dangerous as a necrotic tuberculous gland bordering on the blood stream. In 'malignant rheumatic endocarditis,' the organisms reach the surface of the valve, multiply rapidly, and are discharged into the blood stream. Poynton and Paine find that the organisms from these 2 types of rheumatic endocarditis are so similar as to be considered identical, that the organism from 1 type can produce both types in animals, that these 2 types are forms of 1 and the same disease.

By most authors this conception is not held, the great majority describing as a separate clinical entity the disease known as subacute and chronic infective or malignant endocarditis or endocarditis lenta. The malignant rheumatic endocarditis of Poynton and Paine is undoubtedly the same disease.

Most authors agree with Andrewes and Horder that rheumatism is related to subacute endocarditis only in so far that by damaging the valves it predisposes them to infection by low grade streptococci. They believe that the organisms of Poynton and Paine are agonal invaders or terminal infecting agents, or come from cases of endocarditis lenta. Horder, in 1906, cast much doubt on the validity of Poynton and Paine's assertions. His cultures of the blood in rheumatism were uniformly negative; in chronic "malignant endocarditis," almost always positive. Cultures from rheumatic tonsils and tissue and exudates yielded discordant results. Much doubt is cast on the importance of animal experiments, for saprophytic streptococci from throat and intestine produced arthritis and endocarditis. How is it, he asks, that if simple rheumatism so rarely yields positive blood cultures, for reasons given by Poynton and Paine, that Beaton and Walker obtained 3 positive blood cultures by merely pricking the ear? If the blood contains so many organisms, why do many reliable investigators fail to grow the organisms?

In reply to the criticisms of their work Poynton and Paine offer the fact that patients from whom they isolated the diplococcus have gone on to complete recovery and lived for years afterward, and that, therefore, the organisms cannot be regarded as agonal invaders.

Much support apparently is given to the contentions of Poynton and Paine by the recent writings of Rosenow on the transmutation of organisms[60] and on the etiology of rheumatism.[59] Using semi-anaerobic methods he cultivated organisms from the joints of 14 of 16 cases. From the blood, using the usual methods, he obtained 1 positive result in 3 cases, but by laking the blood before inoculating the mediums he obtained 3 positive cultures of 4 attempts, that is, a total of 4 positive blood cultures in 7 cases. The organisms fall into 3 groups as follows:

1. Five strains. Long chains of diplococci, large clumps in broth, green on blood agar.
2. Six strains. Size of hemolytic streptococci, short chains and diplococci, slight hazy hemolysis on blood agar. This group especially found in the type of rheumatism with muscular manifestations.
3. Three strains. Clumps of micrococci, occasionally short chains and diplococci, small grayish colonies on blood agar.

None ferments inulin, all produce large amounts of acid. In order of virulence come Groups 2, 1, 3 (least). Rosenow has converted one type into the other, and all into typical pneumococci. Organisms of Group 1 on prolonged cultivation come to resemble Streptococcus viridans. In addition, he has converted 3 pneumococci into rheumatic streptococci. Rosenow believes that the organisms of the pneumococcus-streptococcus group may undergo mutation within the body and thus produce different specific pathologic lesions in the body at different times. If this is true, much support is given to the theory of Poynton and Paine.

Up to the present we have not been able to confirm the assertions of Rosenow as to "elective affinity," although we have no doubt that it holds at least to a certain extent (and clinical experience alone would make us give some credence to the view).

In this connection the studies of Rothschild and Thalhimer[73] are of importance. These authors employed for their experimental studies the same organisms described in the present paper. They employed the name Streptococcus mitis, however, for the anhemolytic streptococcus of subacute endocarditis. They produced arthritis in 50% of rabbits injected with endocarditis streptococci and endocarditis in only 7%, results which are the reverse of Rosenow's. This experimental arthritis differed in no way from that reported by investigators of the Streptococcus rheumaticus, nor from the form of arthritis produced by the authors in 45% of rabbits injected with 5 strains of this organism isolated by Poynton and Paine, Beattie, Lintz, and Rosenow. They concluded that "the deduction of a distinct variety or species of streptococcus based upon the power to cause arthritis in rabbits is unwarranted." The studies of Krumwiede and Valentine[35] and of Henrici[24] corroborate the lack of specificity indicated by their studies.

Swift and Kinsella[70] took 85 blood cultures in 58 cases of rheumatism, and obtained 7 positive cultures in 6 cases. Repetition a short time later was negative in all but 1 patient. The bacteriemia was, therefore, fleeting, in sharp contrast to the persistence of bacteriemia in subacute streptococcus endocarditis. The organisms were anhemolytic streptococci, nonencapsulated, insoluble in bile, 2 strains fermenting inulin. The fermentation tests showed no uniformity, but optimum medium was not used for these tests. Joint cultures made in 34 cases proved sterile.

TABLE 6

STREPTOCOCCI FROM CASES OF RHEUMATISM, ISOLATED BY VARIOUS AUTHORS

	Gram Stain and Morphology	Capsule	Glucose Broth
Poynton and Paine 34 From Mus. Nat. Hist.	+ Large; lanceolate diplococci in chains	Pneumococcus type on many	Diplococci; long and short straight chains
Poynton and Paine 34 From Dr. Rosenow	+ Large; lanceolate diplococci in chains	0	Long chains
Beattie 254 From Mus. Nat. Hist.	+ Ovoid coccus; pairs and groups	0	Granular growth; diplococci; short chains; groups
Beattie 254 From Dr. Rosenow	+ Ovoid coccus; pairs and groups	Some 0; mostly with pneumococcus type	Diffuse clouding; diplococci; short chains
Lintz 399 From Mus. Nat. Hist.	+ Lanceolate diplococcus; short chains and groups	Pneumococcus type	Granular growth; diplococci; short chains; groups
Lintz 399 From Dr. Rosenow	+ Large, lanceolate diplococci in chains	0	Granular and mucoid growth; very long chains
Rosenow 735 April, 1913	+ Lanceolate singles; diplococci; short chains; groups	0	Granular growth; conglomerate masses of chains
May, 1913	Unchanged	except	
Rosenow 738a From tonsil April, 1913	+ Lanceolate, singles; mostly diplococci; short chains and groups	Narrow, poorly staining; atypical	Sparse, clumpy growth; long chains; some conglomerate

I have had for study 3 rheumatic cocci obtained from the Museum of Natural History, and the same strains from Dr. Rosenow. These have been studied in the usual manner and the results recorded in Table 6. It is interesting to note that the same organisms from different sources show differences in their characters. This may be due to differences in the nutritive medium on which they have been grown, climate, etc. Thus the museum cultures were kept on serum agar, Rosenow's on blood agar.

The organisms fall into the class of anhemolytic streptococci. They are gram-positive, may possess a typical pneumococcus capsule, form large amounts of acid, as shown by the rapid and intense precipitation, do not ferment inulin, are not dissolved by bile, and on blood plates grow grayish-white with no production of green and no clearing, with the exception of 1 organism (Poynton and Paine 34, Museum of Natural History), which cleared on human blood agar but not on ox-blood agar. The rusty brown or chocolate color on blood agar noted by English observers has not been seen on plain blood-agar plates, but on glucose-blood-agar plates the organisms produce in 48 hours a brownish growth, surrounded by a wide zone of hazy medium, which is partly cleared but precipitated as well. This appearance has not been observed with any other organisms, and is probably due to the intense production of acid from the glucose of the medium.

TABLE 6—*Continued*

STREPTOCOCCI FROM CASES OF RHEUMATISM, ISOLATED BY VARIOUS AUTHORS

Precipitation	Inulin	Bile Test	Human-Blood Agar	Plain Ox-Blood Agar
+++	0	Not dissolved	Grayish-white growth; moderate clearng	Grayish-white; linear clearing
+++	0	Not dissolved	Grayish-white; no clearing	Grayish-white; linear clearing
+++	0	Not dissolved	Grayish-white; no clearing	Grayish-white; linear clearing
+++	0	Insufficient growth	Grayish-white; no clearing	Grayish-white; linear clearing
+++	0	Not dissolved	Grayish-white; no clearing	Grayish-white; linear clearing
+++	0	Insufficient growth	Grayish-white; no clearing	Grayish-white; linear clearing
+++	0	Not dissolved	Clearing	Delicate transparent growth; no clearing
				Grayish-white growth; underlying medium cleared
0 Except occasionally about the condensation water	0	Not dissolved	Dry grayish-white growth	Grayish-green growth; underlying medium green

The organisms are thus seen to differ slightly from the streptococcus of subacute endocarditis. The pneumococcus-type capsule is more frequent in the former and is apparently retained for a long time on artificial medium. They produce large amounts of acid, as evidenced by their intense power of precipitation and by the peculiar growth on glucose blood agar.

Employing our present blood-culture technic and the Rosenow technic, we have never obtained positive results in cases of acute articular rheumatism. Hence the problem of differentiating such organisms from those we have so often isolated in cases of subacute endocarditis has not arisen. If we are confronted by such a problem, I believe that the clinical course and symptoms will have to be our guide in the differential diagnosis. Moreover, the bacteriemia thus far found in cases of rheumatism, is fleeting, while that of subacute endocarditis is very persistent. Positive results in the former apparently require special methods, while in the latter the routine procedure yields a high percentage of positive culture.*

I have also had for study 2 of the organisms isolated by Rosenow, 735 and 738a. Of these, the 1st seemed to be a hemolytic streptococcus, and the 2nd an anhemolytic encapsulated streptococcus which lacked the property of precipitation, that is, an intermediate organism. At any rate neither conformed culturally with the cocci of the other authors.

It must be admitted that no fair conclusions can be drawn from cultural studies made on organisms isolated a long time ago and kept alive on various artificial mediums for long periods of time. We know that such conditions may produce marked changes in cultural and morphologic characteristics, and pathogenic properties as well.

As regards the true significance of anhemolytic streptococci found in the blood of cases clinically considered as instances of rheumatic fever, a final opinion cannot be given at present. Some of these cases are really instances of metastatic arthritis secondary to a definite primary inflammatory or purulent focus. Such cases should be named according to the organism isolated from the primary focus, the joints, or the blood. In clinical medicine cases of arthritis secondary to a primary focus due to streptococcus hemolyticus are now properly termed "streptococcus (hemolyticus) arthritis." It will be important to determine whether the streptococci found in connection with true rheumatic polyarthritis are secondary invaders, a possibility entertained by Swift and Kinsella.

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* Further studies may reveal more definite characteristics for the arthritis streptococci (anhemolytic) than those pointed out in the 2 preceding paragraphs.

SUMMARY

For the differentiation of the organisms of the pneumococcus-streptococcus group for clinical diagnostic purposes, a morphologic and cultural method which has yielded good results has been presented. Based on a study of many organisms according to this method, a classification has been adopted which seems to be as definite as can be attained in our present state of knowledge. There is much evidence that the classification is artificial from a biologic point of view, as shown by the variations within the classes and by the existence of intermediate and transitional forms, as well as by the mutations which have been described.

The limitations of the method are seen in the study of the anhemolytic streptococci. In this class are included bacteria leading a saprophytic existence in the mouth, throat, and intestinal tract of man, bacteria producing local infections in adjoining parts, bacteria producing a bacteriemia, notably when the endocardium is the site of the local lesion, bacteria thought to be the cause of ordinary acute articular and muscular rheumatism, and of simple rheumatic or verrucose endocarditis. The limits of variation within this class of organisms are so wide that their separation into definite types associated with definite pathologic lesions does not at prsent seem possible by this method of study. According to our own experience, the finding of anhemolytic streptococci in the blood stream usually indicates an infection of the endocardium. The organisms from these patients exhibit certain peculiarities which are more or less distinctive, but in every case the laboratory finding serves as in the case of other bacteriemias, to confirm a diagnosis justified by the clinical symptoms. I would suggest that for practical purposes the anhemolytic streptococci from cases of subacute endocarditis be called endocarditis streptococci (the name viridans being misleading), while those organisms isolated from cases of polyarthritis be called arthritis streptococci.

With regard to the bacteriologic method itself, I wish to emphasize certain points. All studies to be of value should be made on organisms freshly isolated from the body for there is sufficient evidence to show that not infrequently they undergo changes when cultivated. Studies should be complete so that more evidence of the existence of intermediate and transitional forms may be established. For the inulin test the Buerger modification of the Hiss medium should be used. Blood-plate studies are especially instructive and should include not only the observation of the original blood-culture plates, but also streak cultures on heterologous human and ox-blood agar plates. It is essential that the agar should be free of glucose in blood-plate studies.

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